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DOCUMENT-IDENTIFIER: US 3911108 A
TITLE: Process of producing bovine milk products containing specific antibodies

ABPL:
An enhanced yield of antibodies specific to an antigen, which neither propagates in the mammary gland of a lactating bovine nor produces in the milk of a bovine any significant quantity of antibodies specific to the antigen, is obtained in the milk of a lactating bovine by inoculating the antigen into the mammary gland of a lactating bovine on at least two consecutive days at intervals during the lactation period of the bovine; the bovine having first been inoculated with the antigen either during its dry prepartum period or during the 24 hour period after parturition. By using such a process a bovine milk product can be produced containing anti-transmissible gastroenteritis antibodies that can be used to protect baby pigs against transmissible gastroenteritis.

BSPR:
In its broadest aspect this invention relates to an improved process of producing bovine milk products containing antibodies specific to antigens that do not propagate in the mammary gland of a lactating cow. In one of its specific embodiments the invention encompasses a process of producing bovine milk products containing antibodies capable of neutralizing living gut-origin transmissible gastroenteritis viruses that cause an acute enteric disease in baby pigs.

BSPR:.
Easterday et al, (1959) in the Am. J. Vet. Res., 819-824, commenting on the work of Mitchell et al., observed that it seemed quite evident, on the basis of their work and the work of Mitchell et al., that a number of viruses cannot only propagate within the mammary gland but can also produce inflammation. Further, the authors were of the opinion that some of the idiopathic cases of mastitis uncovered by practitioners may be the result of a virus infection of the mammary gland pointing out that it was a matter of speculation whether the viruses, if any, would be specific mammary gland viruses or other types of viruses. The authors reported that, in addition to the Newcastle disease virus studied by Mitchell et al., vesicular stomatitis virus and vaccinia virus, when infused into a lactating bovine mammary gland, caused inflammatory reactions that persisted for

varying periods and, in most cases, stimulated the production of considerable neutralizing antibody.

BSPR:
Other experimenters similarly have observed that the innoculation of the mammary gland of a lactating bovine with live viruses causes infection and in some instances a severe reaction leading to a temporary disfunction of the gland. Bannister et al. (1959) in the Can. J. Comp. Med. and Vet. Sci. 23, 47-49, reported that mastitis was produced experimentally in a cow with the virus of enzootic abortion of ewes, a member of the psittacosis-lymphograbuloma group. Greig et al. (1965) in the Can. J. Comp. Med. & Vet. Sci. 29, 57-62 found the bovine udder of non-immune animals was readily susceptible to bovine herpes virus producing an acute limited infection leading to a temporary disfunction of the gland. Straub et al. (1965) in Berl. Munich. tieraerztl. Wschr. 78(20)386-89 reported that the intramammary inoculation of 2 milliliters of freshly harvested CBV-D enterovirus suspension with a virus content of 10.sup..sup.-5 TCID.sub.50 produced acute catarrhal mastitis in the infected quarters which persisted for about 14 days before recovery.

BSPR:
Considering this present state of the art with respect to the stimulation of antibodies in the mammary gland of a lactating bovine, it is a general object of this invention to provide a method of increasing in the milk of a lactating bovine the quantity of antibodies specific to an antigen that does not propagate in the mammary gland of a lactating bovine but is capable of stimulating a measureable antibody response.

BSPR:
Further, specific objects of this invention are the provision of a bovine milk product containing anti-TGE antibodies capable of protecting TGE-susceptible baby pigs, the provision of technically and economically feasible methods of producing such a bovine milk product, and, finally, the provision of a method of protecting baby pigs against TGE employing such a bovine milk product.

BSPR:
These and still other objects and advantages of the invention, which will be apparent to those skilled in the art from the following description, are provided by restimulating the mammary gland of a lactating bovine with an antigen, which does not propagate in the gland but is capable of stimulating a measurable antibody response, on at least two consecutive days at intervals during the lactation period of the bovine, the mammary gland of the bovine having first been prestimulated with the antigen by inoculating the antigen into the gland of the bovine either during its dry prepartum period of during the 24 hour period after parturition.

DEPR:
While any bovine may be used for the practice of this invention, dairy cows will normally be used and particularly those breeds giving the highest yield of milk such as the Holstein breed.

DEPR:
The antigens which may be employed in the practice of this invention may be any substance which, after intramammary injection into the mammary gland of a lactating bovine, (1) does not propagate in the mammary gland, (2) does not cause any significant mastitis reaction in the mammary gland and (3) is capable of stimulating a measurable antibody response in the mammary gland after one to six single intramammary gland injections at intervals of 4 to 10 days. Potentially useful antigens may be any viruses, mycoplasma, rickettesiae, bacteria, fungi, protozoa, proteins, allergy causing substances, and so forth that exhibit these three characteristics when intramammarily inoculated into the mammary gland of a lactating bovine. Illustrative of antigens that may have these three characteristics and that thus may be employed in the invention process are: transmissible gastroenteritis virus, calf scours virus, swine dysentery bacteria, Syncythial virus, Mycoplasma, Vibra cholera bacteria, poliomyelitis virus, Escherichia coli bacteria, Shigella sp bacteria and certain causative agents of respiratory diseases in which protection is associated with locally produced secretory IgA. Any living antigens, such as bacteria, virus and fungi that cannot be used as such because they produce a significant mastitis reaction may be rendered suitable for the invention process by first treating them by methods well known in the art, such as attenuation, inactivation, or detoxification, to neutralize or diminish to an acceptable level their infective and pathogenic properties for the mammary gland care being taken not to significantly alter the original immunogenic characteristics of the living organism. Because living organisms normally generate maximum antibody response, they are preferred for the invention if no significant mastitis reaction is engendered by their use. The antigens are suspended in a suitable liquid medium, such as a sterile physicological saline solution, for the intramammary injections, care being taken to insure that they contain no contaminating substances, that could cause infection of the mammary gland or other extraneous antigens that can diminish the stimulation of antibodies from the antigen.

DEPR:
In determining whether any potential antigen will operate in the process of this invention a quantity of the antigen, dispersed in an aseptic biological solution compatible with the mammary gland of the cow, is injected into each quarter of the mammary gland of a lactating cow in a concentration and volume likely to engender an antibody response but not so great as to cause a mastitis reaction. If the antigen appears to propagate in the gland, as evidenced by the presence of the antigen in the milk of the bovine obtained in the second or later milkings after inoculation, and a sizeable quantity of antibodies are subsequently detected in the milk after the antigen is no longer present, then it would not be a suitable antigen for the practice of this invention. If, however, no virus propagation or antibody response is noted within fourteen days after the first injection then injections of either constant or alternatively of increasing dosages of the trial antigen are repeated at regular intervals

such as 4 to 10 days for up to six inoculations to determine if any antibodies are produced in the inoculated mammary gland as evidenced by the presence of antibodies in the bovine milk. If during the course of giving these trial inoculation shots, a mastitis reaction not attributable to any other cause is observed and the volume and dosage of the shots is not considered to be excessive, or, if after six trial stimulations of either constant or gradually increasing dosages, no antibodies are found in the lactating bovine's milk then it can be assumed that the trial substance will not be a suitable antigen for the practice of this invention. However, if the candidate antigen does produce a measurable antibody response in the lactating bovine's udder, as determined by the presence of antibodies in the milk of the bovine, without showing any signs of propagation and further without causing any significant mastitis reaction, then it could be considered a suitable antigen for the practice of the invention process. While usually only one kind of latent antigen is preferably employed per lactation period, there may be instances when a mixture of antigens may be used to give an immune milk product having more than one type of antibody if trials indicate that this can be done without engendering a mastitis reaction and an acceptable level of antibodies can be obtained. In any event, the same type or types of antigens should be used throughout any one lactation period. Poorer results are obtained if new types of latent antigen are first used after lactation has commenced. Further, if the immune milk product is to be used to assist in protecting animals from disease caused by the organism from which the antigen is derived, then the antigen used should be one that stimulates the production of antibodies which neutralize the organisms as they occur in their natural hosts.

DEPR:
A variety of methods can be used to detect and quantitate the bovine milk antibodies. These methods include in vitro serologic tests in which the reactions between the antigen and milk antibody are observed and recorded microscopically or macroscopically as well as by in vivo neutralization tests. In the latter test, the host animal is the indicator of the test results.

DEPR:
For the multiple consecutive day restimulation procedure of this invention to effectivety produce increased yields of antibodies to the latent antigen, it is necessary that the mammary gland of the cow be prestimulated either during its preparturition dry period or as soon after calving as is feasible and no later than 24 hours thereafter, with at least one and preferably two inoculations of the latent antigen. As the preparturition prestimulation procedure has been found to generate the greatest antibody response in the lactating bovine, it is the preferred method. While one preparturition inoculation into the dry mammary gland will induce increased antibody yield in the subsequent milk of the cow, it is preferred to use at least two and in some instances three or more preparturition inoculations to maximize antibody yield. When more than one preparturition inoculation is employed better results are observed when they are spaced about

one to two weeks apart with the last inoculation being given about one week to three weeks prior to the expected calving date. Also when only one prepartum inoculation is used it also is best administered about 1 to 3 weeks before calving. If the last or only prestimulation is administered within the 6-day period immediately prior to calving, the yield of antibodies in the colostrum and first milk can be reduced for some antigens. Further, if multiple prepartum prestimulations of some antigens are given too close in time, such as 1 to 6 days apart, there can be increased risk of causing damage to the udder.

DEPR:
The multiple restimulations of the mammary gland of the lactating bovine are preferably administered so that there is a time period of 18-26 hours between the inoculations on consecutive days. When less than about 18 hours is utilized subsequent antibody production may be diminished, while when a period of greater than about 26 hours is employed there is a chance of the cow's udder becoming engorged with milk and inflamed, thus making it susceptible to bacterial infection and damage with possible consequent loss of future milk production. As used in the claims the expression "consecutive days" means any series of two or more restimulations administered so that there is about an 18 to 26 hour interval between any two restimulations even though there may be instances where they would be adminstered on the same day when administered less than 24 hours apart or with an intervening day when administered more than 24 hours apart.

DEPR:
The TGE antigen utilized for the prestimulation and restimulation of the bovine, was an Ohio TGE virus isolant propagated in and harvested from 3-day old baby pigs according to the following procedure.

DEPR:
If, however, the dosage of the TGE virus antigen is increased above this quantity and if the bovine has first been prestimulated either during her dry preparturition period or within 24 hours of calving as previously described for the preferred double restimulation procedure, then higher yields of the anti-TGE antibody will be obtained. In particular, when a single restimulation procedure is to be followed, then a significantly greater quantity, such as about 7 to 10 .times. 10.sup.5 LD.sub.50 units or even more, of the TGE virus antigen should be inoculated into each quarter at the time of each single restimulation. When required to prevent undesired udder reactions due to excessive volume, the single inoculation dose may be used in a more concentrated form than the inoculation doses used for the double restimulation procedure so as to reduce to an acceptable level the dose volume.

DEPR:
While an arbitrary 6-day interval was chosen between the series of restimulation inoculations after the second series, it seems obvious from the data plotted in FIGS. 1, 2, 3 and 4 that an even greater production of anti-TGE antibodies could have been obtained by utilizing shorter intervals such as 4 or 5 days

between the series of restimulation inoculations provided they did not cause a immunogenic paralysis or mastitis response in the udder. The desirability of such shorter intervals would, of course, have to be determined by cost analyses to determine if the added value of the increased production of antibodies would more than offset the added cost of more frequent double restimulations. Conversely, such economic analyses could indicate that minimum anti-TGE antibody costs could be achieved by utilizing longer intervals between multiple restimulations, such as seven to ten day intervals or so long as the level of antibodies continued at economically useful levels in the bovine milk.

DEPR:
In Table 2 there is compiled the results of a series of experiments conducted to determine the protective properties of different samples of filtered and decreamed milk, that had been obtained at various intervals after calving from bovine that had been restimulated by the invention method. The data indicates that 32,000 baby pig protective units (BPPU) a day protected all the tested baby pigs from mortality against a challenge with 100 LD.sub.50 of the National Animal Disease Laboratory (NADL) TGE virus while 16,000 BPPU per day protected only 15 of the 24 tested pigs. The results further confirmed that the level of BPPU determined for any sample of bovine milk correlates with the protective effect actually observed when that milk is fed propylactically to challenged baby pigs. Based on this observation, it was concluded that to be effective the quantity of bovine milk fed to baby pigs must be adjusted in accordance with the concentration of baby pig protective units contained in the milk, or, in other words, that the BPPU titer be used as a standard for determining the potency of and hence the quantity required for any given sample or combined samples of the bovine milk fed to baby pigs for propylactic protection.

DEPR:
Based on the results of the experiments compiled in Table 2, two additional series of experiments were conducted to determine the effectiveness of various samples of bovine milk containing anti-TGE antibodies fed prophylactically in a quantity providing 32,000 BPPU daily to each of a number of baby pigs, either kept in isolation or with a nursing litter, Tables 3 and 4 respectively, when each was challenged with 100 LD.sub.50 of the NADL TGE virus.

DEPR:
In Table 3 there is compiled the results of force feeding baby pigs kept in individual isolation units. The pooled first 2 days colostrum and the pooled milk samples obtained from the 3-day periods immediately following the first three double restimulations of cows 10, 359, 102 and 406 were fed to 16 test groups each having four baby pigs. Of the 64 pigs tested only 12, or 18.7% exhibited any morbidity and only five, or 7.8% died, from TGE when protected with the bovine milk containing anti-TGE antibodies as compared to the 100% morbidity and mortality observed in the four control pigs receiving only normal cow's milk.

DEPR:
From Table 4, compiling the results of protective tests for nursing baby pigs supplementally force fed the pooled first 2 days' colostrum and the pooled milk from the 3-day periods immediately following the first three double restimulations of cows 359 and 10, it can be seen that the various milk samples containing anti-TGE antibodies, when fed three times a day in a quantity providing 32,000 BPPU to each pig, protected 43 of 64 tested pigs, or 65.7% against any signs of morbidity and 52 of the same 64 pigs, or 81.3% against death. By comparison of the 31 nursing control pigs supplementally force fed normal bovine milk, only three of the 31, or 9.7% did not exhibit morbidity while only six of the 31, or 19.5% did not die. Additionally all the survivors of the pigs fed the bovine milk containing anti-TGE antibodies grew normally while the pigs fed the normal cow's milk remained stunted.

DEPR:
It is recognized in the field of animal disease that artifical challenges like those used for the tests shown in Tables 3 and 4 often can be more severe than a challenge experienced by baby pigs naturally exposed to the disease. Further, it is recognized that the force feeding of baby pigs, also having available alternative sources of milk, may be ineffective for those baby pigs who have fed to satiation prior to force feeding, because of the propensity of such pigs to regurgitate some or all of the force fed milk. Also, less than the forcefed amount of milk may be retained by baby pigs suffering from other digestive disorders. Finally, not all baby pigs in any sizeable population of tested pigs will be equally healthy, some being more susceptible to TGE viruses than others. All of these enumerated factors, plus others which may also be important, could be contributing to the less than 100% protection observed for the baby pigs described in Tables 3 and 4 that were force fed the invention bovine milk containing anti-TGE antibodies.

DEPR:
It would be obvious that the problem of regurgitating due to satiation or other digestive disorders could be minimized and possibly obviated by making available to baby pigs to be protected, the bovine milk product containing anti-TGE antibodies fortified, if desired, by vitamin, mineral, antibiotic, etc. supplements, so formulated, as for example with sugars or salt, ands dispensed, as for example in self-feeders, that the baby pig would have a preference for and thus consume the calculated amount of the invention milk product required for protection against TGE. Additionally, such a self-feeding system would minimize the cost of administering the invention milk product and provide an ideal way of feeding nutritional or other propylactic supplements to the baby pigs. In case the baby pigs do not consume the amount of immune milk required to give adequate immunity by a self-feeder system of administration, such as might occur in baby pigs up to two or three days of age, then it may be necessary to supplement or even substitute for the self-feeding procedure in these first few days, a force feeding procedure to insure adequate consumption of the TGE immune milk product.

DEPR:
In addition to its propylactic utilization, the bovine milk containing anti-TGE antibodies may also be applied therapeutically for the treatment of TGE, especially when the disease has not progressed too far. During the early stages of the disease, before considerable villus atrophy has taken place, the anti-TGE antibodies present in the bovine milk product of this invention would be expected to effectively neutralize the TGE virus and thus prevent development of the later mortality-inducing effects of TGE. Even piglets suffering from an advanced form of TGE may exhibit a higher percentage of recovery if fed TGE virus-neutralizing quantities of the invention milk products because of the fast villus regeneration observed in young pigs.

DEPR:
Using this test, it can be determined if other living TGE virus obtained from other sources, such as a cell cultured TGE virus propagated under favorable conditions would be a suitable TGE virus antigen for use in the invention process either by itself or in admixture with a live gut-derived TGE virus. When such mixtures are to be used, their suitability as a TGE virus antigen should be determined by feeding the proposed mixture to the pregnant sow as above described to determine if it will have the immunogenic character necessary to stimulate protective anti-TGE antibodies in the mammary gland of a lactating bovine. For brevity, any living TGE virus antigen or antigen mixture having the above characteristics will be referred to as an "immunogenic living transmissable gastroenteritis virus".

DEPR:
When the bovine milk product containing anti-TGE antibodies is to be used as a feed or liquid supplement for baby pigs for protection against TGE, it may be processed as follows to give a sterile milk product free of infective organisms. The whole milk after filtering is passed through a cream separator to give a skim milk product to which there is then added 2 mls. of B-propiolactone per 1000 mls. of the skim milk. This mixture is incubated 2 hours at 37.degree.C. and then stored for at least 24 hours at 4.degree.C. before use. After this treatment, the skim milk tests negative to bacteria, mycoplasma or viruses. This treatment appears to have no effect on the number of BPPU's in the skim milk. Baby pigs fed 27 mls. of the treated milk containing 400 BPPU's/ml. 3 times a day did not show any adverse effects and resisted a challenge of 100 LD.sub.50 of the NADL TGE virus given 48 hours after the start of the feeding program. Alternatively, in place of B-propiolactone there may be used other equivalent chemical sterilants, such as ethylene oxide, propylene oxide and epichlorohydrin described in U.S. Pat. No. 2,705,696, and like sterilants, to free the invention milk products of infectuous microorganisms without significantly diminishing antibody potency.

DEPR:
As employed in the claims, the expression "bovine milk product containing anti-transmissable gastroetneritis antibodies" means

that the product, be it the colostrum or whole milk or the skim milk, whey, or gamma globulin fractions obtained from the whole milk or colostrum, contains antibodies capable of neutralizing living NADL TGE virus by an in vivo neutralization test like that described in Footnote 5 of Table 1.

DEPC:
Preparation of the TGE Antigen Used for Inoculation of the Bovine

DEPC:
Innoculation of Bovine with the TGE Virus Antigen

DEPC:
Assay of the Anti-TGE Antibody in the Colostrum and Milk of Inoculated Bovine

DEPC:
Protective Effect of the Bovine Milk Containing Anti-TGE Antibodies

DETL:
TABLE 1

PRE- AND POST-PARTURITION IMMUNIZATION OF BOVINE WITH TGE VIRUS ANTIGEN.sup.1 AND ASSAY OF ANTI-TGE ANTIBODY IN THE COLOSTRUM AND MILK.sup.2 ANTI-TGE ANTIBODY ASSAY Days Prior to Days After Post Partum Cell Culture Pig Protective Baby Pig Protective Cow Innocula- Parturition of Parturition of day of Titer (In Titer (In Vivo Units (BPPU) per No. tion Site Innoculations Innoculations Milking Vitro Test).sup.4 Test).sup.5 Milliliter of Milk.sup.6

10 IMM.sup.3 34, 22, 8 0 1:640 1:8 800 1 1:320 1:16 1600 2 1:320 1:2 200 IMM.sup.3 6 and 7 8 1:160 1:8 800 9 1:640 1:8 800 10 1:160 1:8 800 IMM.sup.3 14 and 15 16 1:160 1:4 400 17 1:320 1:4 400 18 1:80 1:2 200 IMM.sup.3 21 and 22 23 1:160 1:2 200 24 1:160 1:2 200 25 1:40 1:2 200 IMM.sup.3 28 and 29 30 1:40 1:2 200 31 1:320 1:2 200 32 1:160 1:2 200 IMM.sup.3 35 and 36 37 1:20 1:4 400 38 1:80 1:2 200 39 1:20 1:2 200 359 IMM.sup.3 43, 29, 15 0 1:640 1:16 1600 1 1:320 1:16 1600 2 1:160 1:4 400 IMM.sup.3 6 and 7 8 1:320 1:16 1600 9 1:640 1:16 1600 10 1:640 1:8 800 IMM.sup.3 14 and 15 16 1:320 1:16 1600 17 1:320 1:8 800 18 1:160 1:8 800 IMM.sup.3 21 and 22 23 1:160 1:8 800 24 1:320 1:8 800 25 1:320 1:8 800 IMM.sup.3 28 and 29 30 1:40 1:4 400 31 1:80 1:4 400 32 1:20 1:4 400 IMM.sup.3 35 and 36 37 1:160 1:8 800 38 1:160 1:4 400 39 1:80 1:4 400 102 IMM.sup.3 30, 16 0 1:160 1:16 1600 1. 1:80 1:16 1600 2 1:40 1:16 1600 IMM.sup.3 6 and 7 8 1:160 1:8 800 9 1:320 1:4 400 10 1:40 1:2 200 IMM.sup.3 14 and 15 16 1:20 1:2 200 17 1:80 1:4 400 18 1:40 1:4 400 IMM.sup.3 21 and 22 23 1:40 1:4 400 24 1:160 1:4 400 25 1:160 1:4 400 IMM.sup.3 28 and 29 30 1:80 1:4 400 31 1:160 1:4 400 32 1:40 1:2 200 IMM.sup.3 35 and 36 37 1:80 1:2 200 38 1:80 1:2 200 39 1:20 1:2 200 406 IMM.sup.3 & IM.sup.7 36,8 0 1:320 1:4 400 1 1:320 1:4 400 2 1:160 1:4 400 IMM.sup.3 & IM.sup.7 6 and 7 8 1:80 1:8 800 9 1:160 1:8 800 10 1:80 1:8 800 IMM.sup.3 & IM.sup.7 14 and 15 16 1:160 1:8 800 17 1:80 1:4 400 18 1:40 1:4 400 IMM.sup.3 & IM.sup.7 21 and 22 23

1:40 1:2 200 24 1:80 1:4 400 25 1:80 1:2 200 42 IMM.sup.8 None 5 and 6 7 1:40 No Baby Pig Protection 8 1:40 No Baby Pig Protection 9 1:20 No Baby Pig Protection IMM.sup.8 12 and 13 14 1:40 1:1 100 15 1:20 1:1 100 16 1:10 No Baby Pig Protection IMM.sup.8 19 and 20 21 1:20 1:1 100 22 1:20 No Baby Pig Protection 23 1:10 No Baby Pig Protection 368 IMM.sup.3 42,28,14 0 1:640 1 1:160 2 1:80 5 1:40 IMM.sup.3 6 7 1:80 8 1:80 10 1:40 IMM.sup.3 14 16 1:10 17 1:20 18 1:10 IMM.sup.3 21 23 1:20 24 1:20 26 1:10 IMM.sup.3 28 29 1:10 30 1:10 31 1:20 32 1:10 31 IMM.sup.3 43,29,15 0 1:160 1 1:16 2 1:80 6 and 7 8 1:40 9 1:160 10 1:80 14 and 15 16 -- 17 1:80 18 1:80 21 and 22 23 1:40 24 1:40 25 1:80 28 and 29 30 1:20 31 1:20 32 1:40 35 and 36 37 1:20 38 1:20 39 1:20

.sup.1. TGE virus antigen obtained from the Ohio TGE virus isolant was used for cows 10, 359, 102, 406, 42 and 368. National Animal Disease Laboratory (NADL) TGE virus was used as the antigen for cow 31. The NADL virus was a Miller No. 3 strain of TGE challenge virus obtained from an outbreak of TGE on the farm of Eli C. Miller, Fredricksburg, Ohio in 1965 Suspension of small intestine obtained from a TGE-infected baby pig was passesd thirteen times in primary porcine kidney cell cultures at six to eight day intervals. The thirteenth cell culture passage was filtered through a 0.45 micron millipore filter and was used to infect a germ-free pig. Subsequently, two more passages in germ-free pigs were made, using 0.45 micron filtered suspensions of the small intestines. .sup.2. Milk assayed was strained through a Kendall non-gauze filter pad and decreamed by passage through a DeLaval No. 108 cream separator. .sup.3. 5 milliliters of 10.sup.5 LD.sub.50 units of TGE virus antigen injected into each quarter both pre- and post-partum at a site approximately 11/2 inches above the base of the teat using a polypropylen disposable syringe and a 22-gauge steel needle. .sup.4. The highest twofold serial dilution of decreamed milk with Hanks' Balanced Salt Solution (HBSS) starting at 1:10 (1 volume of milk and 9 volumes of HBSS) that neutralizes an equal volume of Purdue high cell culture passage TGE virus containing 1000 TC-LD.sub.50 per ml. after bein incubated for one hour at 37.degree.C., as determined by the absence of any cytopathic effect seven days after 0.2 mls. of the incubated mixture has been added to a 30 ml. cell culture container containing a primary pi kidney cell culture. .sup.5. The highest twofold serial dilution of the milk with Hanks' Balanced Salt Solution (HBSS) starting at 1:2 (1 volume of milk and 1 volume of HBSS) that neutralizes an equal volume of National Animal Disease Laboratory (NADL) TGE virus containing 100 LD.sub.50 for three-day-old baby pigs per ml. after being incubated for one hour at 37.degree.C., as determined by the absence of any mortality for seven day in two three-day old baby pigs each fed 2 mls. of the incubated mixture. .sup.6. 100 .times. Pig Protective Titer. .sup.7. An additional 5 mls. of 10.sup.5 LD.sub.50 units of TGE virus antigen was also injected intramuscularly. .sup.8. Same as Footnote 3, except only post-partum injections used.

DETL:
TABLE 3 _____ Protective Effect of the Immune Milk.sup.1 for Baby Pigs Housed in Individual Isolation Units against Challenge with TGE Virus.sup.2 No.

Results Cow Post Partum BPPU.sup.3 /ml of of Challenge.sup.4 No. Days Milk of Milk Pigs Morbidity Mortality

10 0-2 1600 4 1/4 0/4 8-10 800 4 0/4 0/4 16-18 400 4 1/4 0/4 23-25 200 4 1/4 0/4 359 0-2 1600 4 0/4 0/4 8-10 1600 4 1/4 1/4 16-18 1600 4 0/4 0/4 23-25 800 4 2/4 1/4 102 0-2 1600 4 2/4 0/4 8-10 800 4 0/4 0/4 16-18 200 4 0/4 0/4 23-25 400 4 1/4 1/4 406 0-2 1600 4 2/4 0/4 8-10 800 4 0/4 0/4 16-18 200 4 0/4 0/4 24-25 400 4 1/4 1/4 Con- trol Milk -- -- 4 4/4 4/4

.sup.1. Bovine immune milk containing anti-TGE antibodies used was filtered and decreamed. It was force fed to the baby pigs three times a day in a quantity providing 32,000 BPPU a day to each pig starting at two days of age and continuing for the following seven days. In addition, normal cow's milk was offered in a milk pan to each pig housed in individual isolation units. .sup.2. Challenge consisted of 100 LD.sub.50 of NADL TGE virus administered orally to each baby pig following the first feeding on the third day of age. .sup.3. Baby Pig Protection Units .sup.4. Based on daily observations of the baby pigs for seven days following challenge.

DETL:

TABLE 4 Protective Effect of the Immune Milk.sup.1 for Baby Pigs Nursing on Non-TGE Immune Sows.sup.2 Against Challenge with TGE Virus.sup.3 Post BPPU.sup.4 No. Partum /ml of Results of Challenge.sup.5 Cow Days of Test No. Milk Milk Pigs Morbidity Mortality

359 0-2 1600 9 3/9 2/9 8-10 1600 5 0/5 0/5 16-18 1600 11 3/11 2/11 23-25 800 8 4/8 2/8 102 0-2 1600 1/6 0/6 8-10 800 8 3/8 2/8 16-18 200 8 3/8 2/8 23-25 400 9 4/9 2/9 Control Non Immune Milk 9 8/9 7/9 Control Non Immune Milk 5 5/5 5/5 Control Non Immune Milk 7 7/7 5/7 Control Non Immune Milk 10 8/10 8/10

.sup.1. Bovine Immune Milk containing anit-TGE antibodies used was filtered and decreamed. It was force fed to the baby pigs three times a day in a quantity providing 32,000 BPPU a day to each pig starting at two days of age and continuing for the following seven days. .sup.2. Normal primiparous sows having no detectable TGE antibody in thei serum or colostrum. .sup.3. Challenge consisted of 100 LD.sub.50 of NADL TGE virus administered orally to each baby pig at three days of age. .sup.4. Baby Pig Protective Units .sup.5. Based on daily observations of the baby pigs for seven days following challenge.

CLPR:

1. A bovine milk baby pig supplement product containing a prophylactically active quantity, effective in baby pigs to withstand a lethal-challenge infection of the pathogen of transmissible gastroenteritis that otherwise typically produces mortality in baby pigs, of anti-transmissible gastroenteritis antibodies comprising the colostrum, whole milk, decreamed colostrum, decreamed milk, whey, whey gamma globulin fraction, or mixtures thereof derived from the mammary gland secretions of a bovine that has been intramammarily stimulated with an immunogenic, pathogenic, or virulent living transmissible gastroenteritis virus, said milk product having been effectively

treated with B-propiolactone or an equivalent chemical sterilant until it tests negative to bacteria, mycoplasma and viruses, and is thereby determined to be free of infectious microoganisms.

CLPR:
2. A method of protecting a baby pig from transmissible gastroenteritis which comprises feeding to the pig the chemically sterilized bovine milk product of claim 1 in a quantity sufficient to prevent the pig from dying from the disease.

CLPR:
3. The method of claim 2 wherein the bovine milk product is fed to the baby pig prior to infection with the disease.

CLPR:
4. The method of claim 2 wherein the bovine milk product is fed to the baby pig after infection with the disease.

CLPR:
5. A method of obtaining a sterilized bovine milk product containing anti-transmissible gastroenteritis antibodies which comprises:

CLPR:
6. The sterilized bovine milk made by the method of claim 5.

CLPR:
7. The method of claim 5 further characterized in that the bovine milk is processed to a decreamed milk, whey or whey gamma globulin fraction prior to being chemically sterilized.

CLPR:
8. The bovine milk product made by the method of claim 7.

CLPR:
10. The sterilized bovine milk product obtained by the method of claim 9.

CLPR:
12. A method of obtaining a sterilized bovine milk product containing anti-transmissible gastroenteritis anti-bodies which comprises:

CLPR:
13. The sterilized bovine milk product made by the method of claim 12.

CLPR:
14. The method of claim 12 further characterized in that the bovine milk is processed to a decreamed milk, whey or whey gamma globulin fraction prior to being chemically sterilized.

CLPR:
15. The bovine milk product made by the method of claim 14.

CLPR:
17. The sterilized bovine milk product obtained by method of

claim 16.

CLPV:
A. prestimulating the mammary gland of a pregnant bovine with an immunogenic, pathogenic, or virulent living transmissible gastroenteritis virus during the period commencing about 60 days prior to calving and ending about 48 hours after calving; and

CLPV:
B. restimulating, after calving, the mammary gland of the bovine with the immunogenic, pathogenic, or virulent living transmissible gastroenteritis virus on at least two consecutive days at intervals during the lactation period of the bovine;

CLPV:
C. milking the bovine after calving to obtain milk containing anti-transmissible gastroenteritis antibodies;

CLPV:
E. each of said mammary gland prestimulating and restimulating steps and being effected by aseptic injection of the transmissible gastroenteritis virus into each quarter of the mammary gland of the bovine reaching the cistern of each quarter.

CLPV:
A. prestimulating the mammary gland of a pregnant bovine with an immunogenic, pathogenic, or virulent living transmissible gastroenteritis virus during the period commencing about 60 days prior to calving and ending about 48 hours after calving; and

CLPV:
B. restimulating, after calving, the mammary gland of the bovine with a single dose of the immunogenic, pathogenic, or virulent living transmissible gastroenteritis virus at intervals of 4 or more days apart during the lactation period of the bovine;

CLPV:
C. milking the bovine after calving to obtain milk containing anti-transmissible gastroenteritis antibodies;

CLPV:
E. each of said mammary gland prestimulating and restimulating steps and being effected by aseptic injection of the transmissible gastroenteritis virus into each quarter of the mammary gland of the bovine reaching the cistern of each quarter.